1989 Probe Study to Determine the Exposure of Aerial Application Flaggers to Organophosphate Pesticides Via Urinary Metabolite Monitoring

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HS- 1568
December 13, 1991

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SUMMARY

Urine from aerial application flaggers was analyzed to evaluate the feasibility of using morning voids to determine absorbed daily dosage of organophosphate pesticides (OPs). Spot samples were also provided by pilots for reference. Each worker was expected to provide one sample as a control following a day with no exposure and three consecutive samples following shifts where OPs were applied. Detailed sample information sheets were supplied by each worker. Urinalysis provided levels of six dialkylphosphate metabolites of the four OPs applied by the application crews (chlorpyrifos, diazinon, malathion, dimethoate). Urine volumes were corrected based on 24 hour creatinine excretion.

Of the 36 workers monitored during the study, only ten provided complete data sets. Of these ten sets of data representing 40 samples, only five samples demonstrated exposure above the exposure estimated using the limit of detection (3.8 ug/kg/day ethyl+methyl metabolites, for an analytical MDL of 25 ppb). For all flaggers the highest absorbed daily dosage was calculated based on one sample not included in the above 40 to be 4.3 ug/kg/day (ethyl metabolites). This corresponds to a potential daily dermal exposure (outside clothing) of 69 mg/day. For pilots the highest one day dosage was 14.2 ug/kg/day (ethyl +methyl metabolites) for a potential daily exposure of 170 mg/day.

Compliance with sample and paperwork submission in this probe was poor. Additional work will require investigators on-site to manage the collections and paperwork at the time of submission.

INTRODUCTION

Exposure of aerial application flaggers to organophosphate (OP) pesticides has not been accurately or adequately measured. Few studies have been done to characterize the potential hazard to which this group of workers is subject. Dermal patch (passive) dosimetry has been the traditional method used to evaluate exposure for the agricultural workplace, but has recently been demonstrated to overestimate daily dermal exposure (Maddy et al., 1989). Urinary monitoring for pesticide metabolites provides excellent indications of absorbed dose, but only when pharmacokinetics are understood and total 24 hour eliminations are collected can the most precise estimates be assured. The resulting exposure data can then be used with greater confidence for risk assessment and management/mitigation applications.

The collection of 24 hour eliminations in the agricultural workplace, however, is well known to be difficult. It was therefore decided to use morning voids to estimate absorbed daily dosages for a small number of flaggers in California. The use of spot samples corrected for 24 hour creatinine can be used with some degree of confidence to calculate absorbed daily dosage. Discussion of the proper method of adjustment and the precision of the results are still in debate in the literature (Edwards and Whyte, 1959; Barber and Wallis, 1986; Franklin et al., 1986; Greenberg and Levine, 1989). However, for the intended use of the results of this and future study the current precision is adequate.

Recent labeling changes initiated by the U.S. Environmental Protection Agency (EPA) eliminating the use of human flaggers for aerial applications of ethyl and methyl parathion have caused concern for various crops grown in the San Joaquin and Imperial Valleys of California. During peak OP use periods applications of parathion must be made at night for reasons of reducing heat stress for application personnel, safety to field workers and better application conditions including less thermal lift and less evaporation of the pesticide before it hits the plants. There is presently no way to eliminate the use of human flaggers at night since substitutes have not yet been adopted, however, EPA granted a one year exemption for 1990 to use human flaggers with parathion application with strict protection requirements while replacements were being developed (Conroy, 1989). Elimination of human flaggers is expected to increase production costs by requiring the use of more costly, less effective alternates that may require more applications and result in reduced numbers of jobs for seasonal and permanent agricultural workers. EPA views flaggers involved in parathion applications to be at significant risk but their surrogate database is limited (Maddy, et al., 1982, Peoples et al., 1979, and Atallah et al., 1982; see Lunchik, 1988) and may be skewed by one study (Atallah et al., 1982) with unusually high exposure measured via passive dosimetry.

The purposes of this probe study were to test the feasibility of spot sample/exposure assessment viability, and to show that a more rigidly controlled study patterned after this probe study may alleviate EPA's concerns for the health of parathion flaggers. It is expected that a final study could be completed this season and provided to EPA by September, 1990.

MATERIALS AND METHODS

Subject Selection

Four aerial application firms were approached in July and without reservation agreed to allow workers to be interviewed. An additional three firms were approached in August/September with the same results. At each location, the study was explained to the workers being asked to cooperate, being translated into Spanish when needed, and each worker who agreed to cooperate was given an informed consent form, which included a request for some personal information, to sign (see protocol, U.C.S.F. CHR Approval No. H6672-05384-01). Workers were selected by

crew rather than by any other criteria. In some organizations, one or more crews are normally assigned to OP application and only these crews were approached to obtain cooperators.

The purpose of taking urine samples required detailed reassurance, surprisingly more so in the case of some flaggers rather than pilots, that the samples to be taken would be used for no purpose other than to assess pesticide exposure.

Procedure for Identification

The last four digits of each cooperator's Social Security Account Number were to be provided on the signed informed consent form. When each sample was surrendered, the bottle was to be clearly marked in indelible pen with this number and the date. Any subsequent referral to the sample or the individual was to be by this number only. CDFA holds the signed informed consent form in security and is therefore the only entity with access to cooperator's names.

Time and Location

From July to October, 1989, personnel from the California Department of Pesticide Regulation, Worker Health and Safety Branch (CDPR, WH&S) and the California Agricultural Aircraft Association (CAAA) collected 98 urine samples representing 28 flaggers and eight pilots employed by seven aerial pesticide application firms located in the Sacramento, San Joaquin and Imperial Valleys. Pilot samples were collected for comparison with flagger data. This period of sample collection is <u>not</u> peak OP use time in any of the locations where workers were monitored.

Method of Sample Collection and Storage

One sample was to be collected from the first void of a morning following a day off (presample) and an additional sample was to be collected each morning for the next three consecutive days following shifts where OPs were handled. In the earliest efforts, urine samples were to be brought in on the day of collection in either the frozen state or to be frozen at the time of submission. Sample data sheets were to be submitted simultaneously. In the latter portion of the study it was determined that an investigator should be on site to receive the samples and paperwork and assume the responsibility for sample storage and care. Urines were held at -20 degrees C until they were analyzed two to three months later.

Method of Extraction and Analysis

Four OPs were applied during monitoring; the ethyl phosphate metabolite parents chlorpyrifos and diazinon and the methyl phosphate metabolite producing parents dimethoate and malathion.

The method used for the extraction and quantification of the ethyl and methyl dialkylphosphate metabolites was essentially that used by Weisskopf et al. (1988) with slight modifications devised by Weiskopf and Seiber in an unpublished work. The method involves the solid phase extraction of four milliliters of the urine followed by a 10% acetone in hexane wash and elution with 20% methanol in acetone. Following dehydration, the sample was derivatized with a butylating agent and quantified by gas/liquid chromatography using a flame photometric detector.

Six alkylphosphate metabolites were detectable in the urine samples at an MDL of 25 ppb. The ethyl metabolites were diethylphosphate (DEP), diethylthiophosphate (DETP) and diethyldithiophosphate (DEDTP). The methyl metabolites were dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP).

Method recoveries ranged from 98.9 $\pm$ 11.8 percent to 119.7 $\pm$ 9.0 percent. Reported values were therefore not corrected for recovery.

Creatinine Analysis

Urines found to have measurable metabolites were delivered to Roche Biomedical Laboratories in Sacramento for creatinine analysis. The method used is still the Picric Acid Color Reaction (Folin, 1914).

Exposure Calculations (Spreadsheet)

Exposure data were analyzed using the spreadsheet WingZ on an Apple Macintosh II computer. Two spreadsheets were created; the first being qualitative to evaluate compliance with regard to sample and data sheet submission in order to select sample sets to be further evaluated. The second spreadsheet was used to calculate exposures for the ten flaggers submitting complete data sets and for the expected exposure calculated using the analytical Limit of Detection. The highest one-day exposure based on the one highest sample each for flaggers and for pilots was also included for comparison.

The first spreadsheet was largely self-explanatory with the primary emphasis being placed on columns indicating sample submission compliance and columns indicating whether the required paperwork for those samples had been turned in. An additional column indicated which workers submitted essentially complete data sets (the absence of paperwork for the presample was overlooked as it was assumed that no exposure had occurred on the prior shift).

The second spreadsheet and calculations can be explained as follows:

Column A presented the last four digits of the worker's Social Security Account Number (a zero preceded two three number entries);

Columns B,C,D represent the weight as reported on the informed consent form, the corrected weight which is weight in pounds minus self-estimated pounds overweight also found on the informed consent form and the weight factor (WF) which is B/C respectively (presently, the weight factor has not been used);

Column E indicated whether the sample was a presample with no exposure or one of the three consecutive samples taken after an exposure period;

Column F was the gross weight in grams of the urine sample plus the container;

Column G presented the sample volume calculated by the equation:

$$G\ ml = (F\ g - 64.9\ g\ tare)\ 1.02\ sp.\ gr.\ urine + 4\ ml\ (removed\ for\ analysis)$$

Column H and I reported the sum in ppm of the three ethyl metabolites and the three methyl metabolites, respectively;

Column J was the quantity of creatinine in mg/dl in each of the positive urine samples;

Columns K and L presented the total alkylphosphate/day in ug for the ethyl metabolites (K) and the methyl metabolites (L) corrected for the amount of creatinine excreted/day in mg via the expression (for the ethyl):

K ug/day = (H ppm/ (J mg/dl creatinine X 0.01 mg/dl)) X 1532 mg creatinine/d

Column M was simply the sum of K + L or the total alkylphosphates (ethyl and methyl) in the urine for the day in ug;

Columns N,O and P presented the total time working with the ethyl organophosphate (chlorpyrifos, diazinon), the methyl organophosphates (malathion, dimethoate) and the sum of the two (time working is primarily the time the airplane was over the field, when two or more OP's were applied simultaneously, the times were treated as additive rather than concurrent i.e. 10 min applying two OP's equaled 20 minutes application time);

Columns Q and R were the result of the division of the mean molecular weight of the parents found in the sample by the mean molecular weight of the appropriate ethyl or methyl metabolites. For a sample where both ethyl parents had been applied the equation would be (chlorpyrifos, MW 351 + diazinon, MW 304)/2 over (DEP, MW 154 + DETP, MW 170 + DEDTP, MW 186)/3 or

327.5/170 = 1.93

with the same true for the methyl parents with the exception of malathion. Malathion's molecular weight having a correction factor of 5X applied as only 20% of the the metabolites of malathion would be excreted as dialkylphosphates (Mattson and Sedlak, 1960), the appropriate numbers for column Q would be;

diazinon/DEPs = 1.79
chlorpyrifos/DEPs = 2.06
diazinon + chlorpyrifos/DEPs = 1.93
malathion X 5/DMPs = 11.7
dimethoate/DMPs = 1.62
(malathion X 5) + dimethoate/DMPs = 6.66

Columns S and T used columns Q and R to calculate the absorbed daily dose in micrograms/day for the ethyl and methyl parents following the equations S = K x Q and T = L x R or

(MW parent/MW metabolite) ug metabolite = ug parent (assumed 100%)

Columns U and V were then the daily dermal exposure under clothing in micrograms/day for the ethyl and methyl parents following the equation U = S/Dermal Absorption and V = T/Dermal Absorption where the appropriate dermal absorption percentages are;

diazinon = 20% (est.)
chlorpyrifos = 3% (Nolan et al., 1973)
diazinon + chlorpyrifos = 11.5% (calculated from est.)
malathion = 8% (Feldman and Maibach, 1974)
dimethoate = 20% (est.)
malathion + dimethoate = 14% (calculated from est.)

Columns W and X presented the potential daily dermal exposure in milligrams/day following the equations W = (U/1000) x 10 and X = (V/1000) x 10 or for example;

mg/day = (ug/day/1000 ug/mg) x 10 (protection from clothing)

Column Y was the sum of columns W + X;

Column Z was the conversion of the worker's weight in pounds to weight in kilograms following the equation Z = B/2.2;

Column AA was the result of the division of columns S + T by column Z or

ug/kg/day absorbed daily dosage = total parent ug/day / weight in kg

RESULTS AND DISCUSSION

The greatest exposure for a flagger for one day's sample came from a day one exposure sample which resulted in an absorbed daily dosage of 4.3 ug/kg/day (ethyl metabolite). This translates to a daily dermal exposure under clothing of 4.1 mg/day and a potential daily dermal exposure of 69 mg/day. By comparison, the highest exposure for a pilot was from a day three exposure sample and resulted in an absorbed daily dosage of 14.2 ug/kg/day (ethyl + methyl metabolite) which is approximately 330% higher than that of the high flagger and 370% higher than the exposure for flaggers calculated at the Minimum Detectable Level of 3.8 ug/kg/day (ethyl + methyl). The daily dermal exposure under clothing for the high pilot sample was calculated to be 14.3 mg/day and the potential daily dermal exposure (outside clothing) was 170 mg/day.

Only ten (28%) of the 36 workers monitored provided complete sets of data which included the presample and three samples for the exposure periods, a data sheet for each of the four samples and a signed informed consent form that included required personal information. None were pilots. Of the 40 samples submitted by these ten flaggers, only five had detectable levels of metabolite above the method MDL.

Eight of the ten workers were monitored by CAAA. The CAAA remained on-site in the Imperial Valley and collected samples on a daily basis. This procedure must be used in follow-up studies. Data sheets were not collected with the samples in most cases including the Imperial Valley, and trying to reconstruct daily application records, most notably times for each application, proved so time consuming that some firms were unable to devote the time required. It should be noted that it is an added responsibility for the firms to manage sample collection and storage and for a busy firm it cannot be expected to be a high priority during the busy spray season.

U.S. EPA has previously used three studies in their surrogate database to calculate expected flagger exposure to parathion. A comparison between our data for absorbed daily dosage and the U.S. EPA assumption can be made using the following calculation (After Ross, 1989):

Assumptions:

6 hour day flagging
Application rate is 2 lb a.i./ac
External exposure is 3.2 mg/hr (Lunchik, 1988)
Dermal absorption rate is 20% (est.)
Work clothing penetration is 10%
Worker weight is 70 kg

$$\frac{6 \text{ hr } (3.2 \text{ mg/hr}) (2 \text{ lb/ac}) (20\%) (10\%)}{70 \text{ kg}} = 10.9 \text{ ug/kg/day}$$

This result is nearly three fold higher than our highest flagger absorbed daily dosage of 4.3 ug/kg/day.

Table 1- Comparison of Exposures for EPA Surrogate Data and CDFA Data From Urine Monitoring

	ADD[a] ug/kg/d	DDE[b] mg/d	PDDE[c] mg/d
EPA Flagger [d]	10.9	3.8	38.4
CDFA Flagger MDL	3.8	4.1	41.1
CDFA High Flagger	4.3	6.9	68.5
CDFA High Pilot	14.2	17.1	171.7

Meinders, et al.,1990

a Absorbed Daily Dosage
b Daily Dermal Exposure (under clothing)
c Potential Daily Dermal Exposure
d Calculated for parathion at 20% dermal absorption

The present study estimated absorbed dosage from a single daily morning urine sample taken 6 - 16 hours after exposure. Weisskopf et al. (1988) have shown that there is an excellent correlation between urinary monitoring for alkylphosphate taken at the end of the work day and dermal dosimetry during ground application of diazinon. Maximal excretion of metabolites from dermally absorbed organophosphates occurs from 8 - 48 hours post exposure indicating gradual absorption of a dermally applied OP (Feldman and Maibach, 1974). A morning urine sample, when corrected for the day's excretion using creatinine as the endogenous internal standard will reflect the protracted absorption interval that protracted urinary excretion suggests.

CONCLUSIONS

1. Comparison between our data and U.S. EPA's surrogate data shows good agreement. We expect that additional study under more controlled conditions could demonstrate a greater margin of safety for parathion flaggers than is presently assumed by EPA. Note that only three of the ten flaggers who completed all sample and data requirements had samples with measurable metabolite levels.

2. We believe that this probe study shows promise for the use of spot samples for measuring absorbed daily dosages, not only for flaggers and pilots, but for other agricultural workers as well, when pharmacokinetics for the organophosphate prove adaptable to this method.

3. It will be absolutely imperative that in any future work on this exposure study, personnel must be present to receive and care for samples on a daily basis. Investigators must ensure that sample paperwork is completed and submitted at the time urine samples are collected.

4. The future study will need to be timed with peak OP use. In the San Joaquin Valley this would begin in mid to late June. Agreement for assistance has already been pledged by one of last year's firms and, if this season is as busy as most, several crews may be available. It may therefore be possible to obtain a more complete study with the cooperation of perhaps only two firms.

5. It would be desirable to reduce the number of OP materials applied and preferable to only obtain samples for application personnel involved with organophosphates for which complete pharmacokinetics are known, eg. chlorpyrifos, malathion or parathion. This may also be achieved by working with fewer cooperators.

6. It was necessary to rely too much on the ability of the application firms themselves to manage sample and data sheet collection and storage which is an added difficult responsibility for the cooperating firm not used to such activities. When this was realized to be the case, plans were made for CAAA to be on-site in the Imperial Valley and to collect samples each day from the cooperating firms. However, sample data sheets were not collected daily and had to be reconstructed after the fact which was not possible for some firms.

ACKNOWLEDGEMENTS

Appreciation is extended to the seven application firms that cooperated with the data gathering and to the flaggers and pilots who were excited by the project. Thanks go specifically to Charlie Jarvis for his patience and to H. Fong, D. Haskell and M. Dong.

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